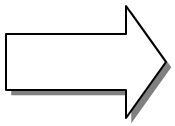
Preliminary Conclusions

- Target Selection
  - Literature based?
  - Expression based?
  - Mass Spectrometry based?
  - Proposal based?
- Affinity Reagent platform selection
  - Are antibodies really the best?
  - Monoclonal vs. polyclonal?
  - What about other scaffolds/platforms?
  - What should we use as the determinants?
    - Experience
    - Available secondary reagents
    - Intellectual property consideration
  - Should we pick a platform or just let everyone play?
- Antigens
  - Should these be produced by each affinity reagent producer?
  - Should these be made as a centralized resource?



Target Selection

- Depends largely on the project's overall goals

- User requested:
  - Focused collections of proteins, networks, pathways of interest to motivated individuals in the community:
    - Many of these pathways have proven themselves to be critical to disease and possibly biomarkers
    - Guarantees that there will be users who will employ antibodies

  But…
  - There are already mechanisms in place to obtain antibodies for these proteins
- Project Design:
  - Centralized process (could involve as many sources as needed) to select antigens for a specific purpose
  - Acknowledges the goal to make affinity reagents for <u>infrequently</u> studied proteins
  - Could be based on:
    - Literature mining – several sources
    - Specific data that indicate likely candidates for specified purpose
    - Abundance data – e.g., look at proteins that are rare in serum
    - Predicted properties of proteins – potential solubility, extra-cellular domains, likely to be secreted
  - Avoid duplication with existing antibodies!

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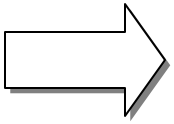

- Reagent Platform
  - Major bottlenecks are reagent platform independent:
    - Making antigen
    - Validating affinity reagent
  - Antibodies are still the most mature technology – tens of thousands already available
  - Alternative platforms show promise but still need further development
  - Monoclonals cost more because individual clones must be screened, but have the advantage that they are a renewable resource
    - This is important because once the reagent is validated it becomes much more valuable
  - Polyclonals cost less and could be very powerful for screening candidates
  - Working with a consistent platform would simplify high throughput (proteome scale) applications
  - Current producers can make between 500 - 1500 monoclonals per year

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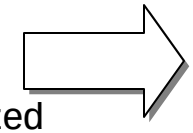
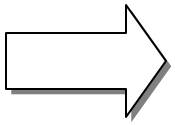


- Antigens
  - Making antigens is a key bottleneck
  - They are useful in both production and validation phases
  - Affinity reagent producers would like a central source for antigen
  - Success rates for affinity reagents depend heavily on the ability to make good quality antigen
  - Not clear how to organize and manage a centralized source for antigen

Preliminary Conclusions II

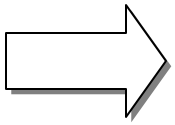
- Mechanisms for contracting the production of the reagents
  - Should we worry about avoiding duplicating effort?
    OR - Duplication can be good and done at the risk of the producer
  - Should we pay only for finished antibodies?
    - Must meet established criteria
    - Validated for pre-selected applications
    - Once acquired, the target gets crossed off the list
  - Should we contract each producer in advance to make a target list of antibodies?
    - Milestones must be met to complete payment
  - Should this effort focus primarily on a centralized mechanism for validating affinity reagents and storing this information?
    - Would a "seal of approval" and the opportunity to list the reagent in this "central database" be an incentive to develop these reagents?



- Production Process
  - Validation is the most important part of this process.
  - Creating a database that tracks various reagents, lists their qualities, and "certifies" them somehow is highly desirable (more details below)
  - Commercial mechanisms are in place to produce antibodies to <u>popular</u> proteins – companies are happy to take these suggestions
    - Some will partner with academics to make these antibodies at low or no cost
  - Incentives are needed to get producers to create antibodies to <u>infrequently</u> studied proteins.
    - Paying for production would ensure that NCI owned the antibodies
    - Paying for validation – i.e., providing it via central source – might induce production of some antibodies
    - "Suggesting proteins" might stimulate some production (but if the proteins are not frequently studied – this may not get traction)

Preliminary Conclusions III

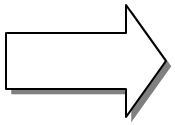
- Distribution
  - Option 1: Centralized repository/distribution center
  - Option 2: Centralized database/producers handle their own distribution through standard commercial mechanisms
  - Both? Other?
  - IP considerations for distribution – limits?
  - If affinity reagents are contracted and paid for by NIH, who owns the IP rights to the reagents?



- Distribution
  - Centralized Distribution has the advantages that:
    - Antibody production and distribution can be handled with consistent standards regarding QC/QA, antibody concentration, etc.
    - Having all the affinity reagents in one location will simplify the development of high throughput applications
    - Simplify the MTA and IP morass
  - Centralized distribution has the disadvantages that:
    - Requires infrastructure
    - Duplicates an existing network
    - Realistically could only apply to reagents produced and paid for by a centralized effort.

Preliminary Conclusions IV

- Validation
  - Which assays should be validated for?
    - Denatured protein
      - Western blot
      - ELISA
      - Immunohistochemistry
    - Native protein
      - Immunoprecipitation
      - Antibody arrays
      - Other…
  - How many affinity reagents needed per target?
  - Which criteria must be met to accept an antibody?
    - E.g., if an antibody is very good at only one of the above, is this ok?
  - Should validation be done at centralized facilities?
    - Or – is it ok for various centers to validate using the same criteria?

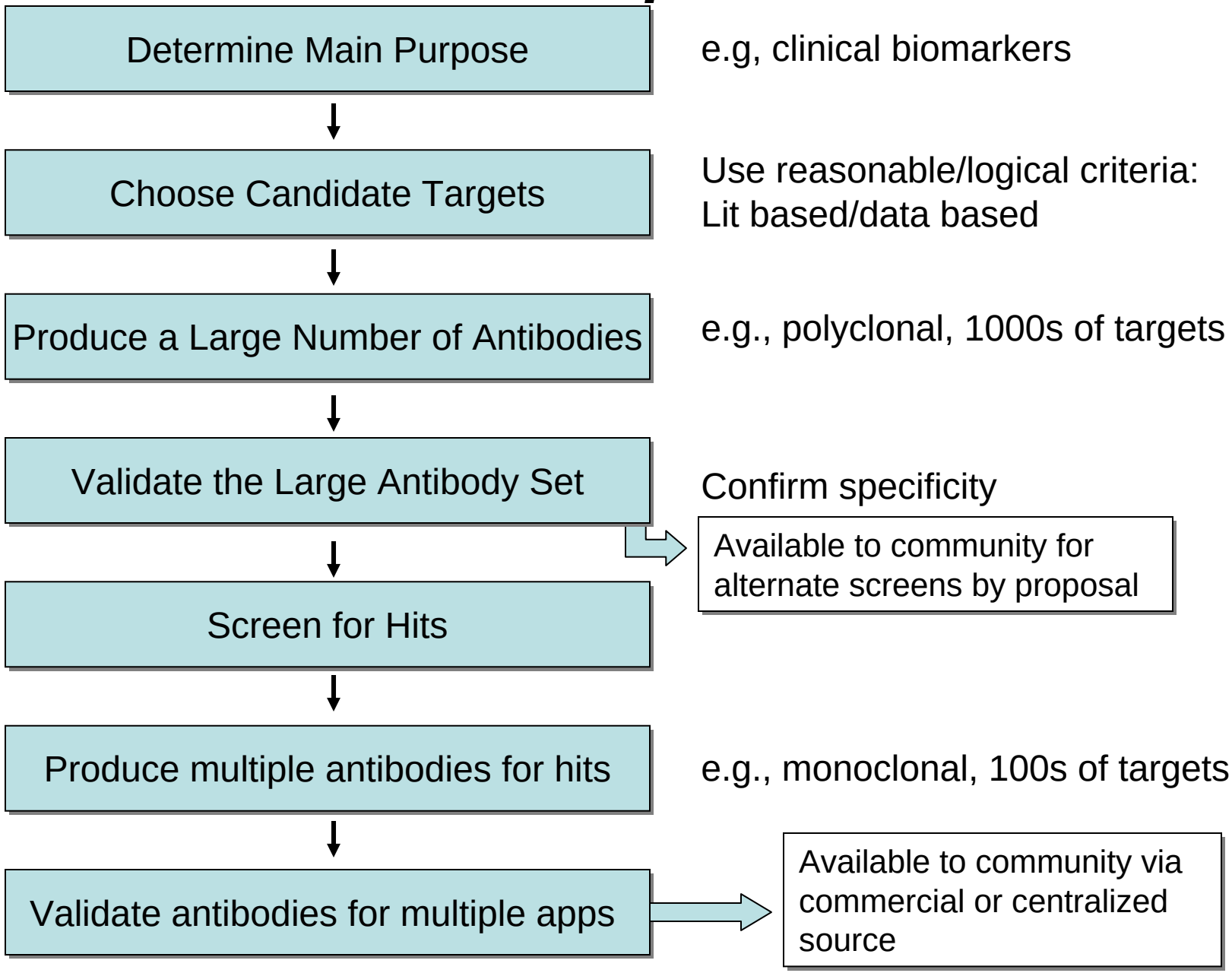


- Validation
  - Strong support for a centralized database that lists the characteristics and qualities for all antibodies, including existing ones
    - Data from existing antibodies could be provided voluntarily by labs using them
      - Specific criteria/format vs. at the labs' own discretion
  - A centralized validation process is needed for reagents generated under a planned project.
    - Consistent standards and SOPs are applied here
    - Data is centralized and qualifying reagents could be "certified"
    - Would need to periodically revisit all reagents to monitor QA/QC
  - Applications to validate include:
    - Standard applications like westerns, ELISA, etc. – usually done as part of antibody development
    - Applications that foster high throughput uses

Straw II

- Proposal
  - Select a single key motivation for developing the reagents
    - i.e., clinical biomarkers
  - Select targets based on reasonable criteria
    - Literature/biology based
    - Existing relevant data
  - Produce a large number of candidate antibodies to the targets, e.g., polyclonal
    - Available to community on a proposal basis
  - Centralized core validation confirms specificity
  - Stage 1: Screen all the antibodies for the key application
  - Stage 2: Take all the “hits” and produce multiple antibodies to each, e.g., monoclonal
  - Both centralized and distributed validation of monoclonals
    - Available to community via central or commercial network

Straw Proposal II



Straw II

- Assumptions
  - It's expensive to make and validate multiple antibodies to each protein – this limits the number of antigens that can be targeted
  - Target selection depends on the proposed use for the targets
  - A main thrust of this project is to develop reagents to proteins *not frequently studied already*
    - *There are already strong commercial motivations to make antibodies to frequently studied proteins*
  - At least one main thrust of this project is to find new clinical biomarkers for detecting/categorizing disease from serum

Straw II

- Advantages
  - Focuses on proteins not currently studied
  - Many more first stage antibodies can be made
  - Opportunity to screen for many “hits”
  - Monoclonals only invoked on “interesting proteins”
  - Reduces money spent on less interesting proteins
- Disadvantages
  - Focuses on only one main application
  - Polyclonal antibodies not renewable
  - Proteins that are not hits in this assay might be important in another